c-myc and *c-myb* Oncoproteins During Induced Maturation of Human Myeloid and Erythroid Leukaemic Lines

M. A. Bains, P. Pedrazzoli, T. G. Hoy, and A. Jacobs

c-myc and c-*myb* mRNA have been found to be tightly regulated during haemopoietic differentiation [1]. While the expression of both oncogenes is induced in cells stimulated to proliferate, c-*myc* in the transition from G_0 to G_1 [2], and c-*myb* in the transition from G_1 to S phase [3], expression ceases in terminally differentiating haemopoietic cell lines. This takes the form of a progressive monotonic mRNA decline during macrophage (MΦ) and granulocyte (GN) differentiation and a biphasic mRNA decline during erythroid (E) differentiation [4–6]. Although this decline may reflect the cessation of proliferation which accompanies the differentiation process, recent reports that constitutive expression of either oncogene inhibits differentiation in murine [7] and human [8] leukaemic lines imply a causative role for each in haemopoietic maturation.

We have studied nuclear c-*myc* and c-*myb* proteins through the cell cycle during MΦ, GN, E and megakaryocytic (MK) differentiation of KG1, HL60 and HEL leukaemic cells. The $p62^{c\text{-}myc}$ and $p75^{c\text{-}myb}$ content of propidium-iodide stained nuclei was quantitated by flow cytometry using fluoresceinated antibodies CT14-G4 and MB4.3, respectively (gifts of G. Evan, Cambridge, UK), following our published method [9].

Figure 1 is a dot display of $p62^{c\text{-}myc}$ and $p75^{c\text{-}mcb}$ fluorescence in uninduced HL60, demonstrating a less than two-fold increment in both oncoproteins over the cell cycle.

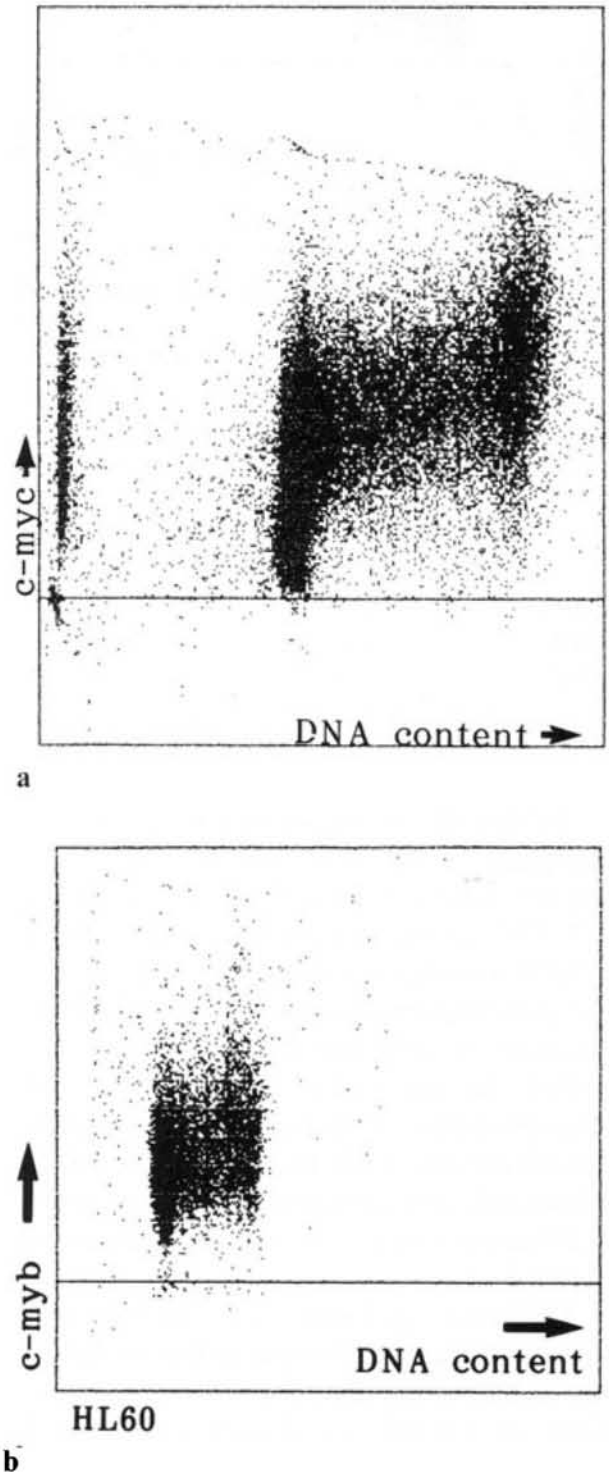

Fig. 1. Dot display of $p62^{c\text{-}myc}$ (**a**) and $p75^{c\text{-}myb}$ (**b**) fluorescence in uninduced HL60 in terms of DNA content

Department of Haematology, University of Wales College of Medicine, Cardiff, UK

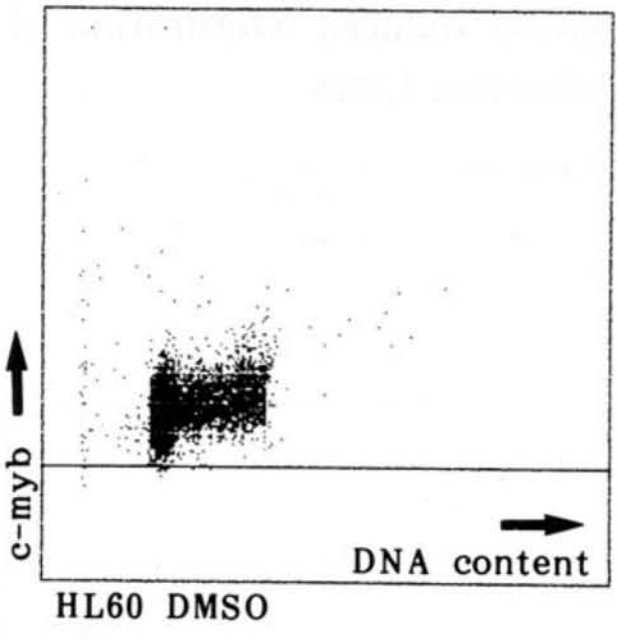

Fig. 2. Reduced level of $p75^{c\text{-}myb}$ in HL60 with DMSO-induced maturation but unchanged cell cycle distribution

Table 1. Oncoprotein levels of HL60, HEL and KG1

Cells	$p62^{c\text{-}myc}$	$p75^{c\text{-}myb}$
HL60	1.0	1.0
HEL	0.9	1.1
KG1	0.68	0.9

Table 1 shows the oncoprotein levels of different leukaemic lines relative to HL60. Figure 2 demonstrates that while $p75^{c\text{-}myb}$ levels declined in HL60 with DMSO-induced maturation, the cell cycle distribution did not change. MΦ induction of different leukaemic lines resulted in an early increase in both oncoproteins, followed by a decline simultaneous with the reduction of S-phase cells and appearance of α-naphthyl-acetate esterase positive cells, as shown in Table 2.

Different patterns of oncoprotein change were found when different inducing agents were used for GN differentiation of HL60, as shown in Table 3. Growth arrest and phenotypic maturation preceded a decline in $p62^{c\text{-}myc}$ in retinoic acid treated HL60; the opposite occurred in DMSO-treated cells.

Hemin-induced E differentiation of HEL resulted in biphasic $p62^{c\text{-}myc}$ and $p75^{c\text{-}myb}$ kinetics, without significant change in growth fraction, while DMSO-induced MK differentiation caused an early and steady decline of both oncoproteins, as shown in Table 4.

Thus although there are early transient increases, generally c-*myc* and c-*myb* proteins decline with differentiation, well before proliferation ceases in some lineages. The kinetics of oncoprotein decline resemble closely those published for the mRNA of these oncogenes. The patterns of decline differ between the two oncogenes and vary with the lineage induced and with the inducer used. There is no simple relationship of either oncogene to proliferation during induced maturation, and the cell cycle distribution of their proteins does not change during the differentiation process. These data support disparate roles for c-*myc* versus c-*myb* during human haemopoietic differentiation and suggest that multiple signal transduction pathways exist for down-regulation of these genes.

In summary, we have quantitated, for the first time, c-*myb* protein over the cell cycle in human haemopoietic cells, using our original method of flow-cytometric assay of nuclear bound c-*myc* protein product [9]. As for c-*myc* mRNA, c-*myb* RNA has been found to be tightly regulated during haemopoietic differentiation, and aberrant expression has been related to failure of maturation induction in both human and murine leukaemic cell lines [1, 7]. We have considered in detail the changes in c-*myc* and c-*myb* proteins during the induced maturation of human myeloid and erythroid leukaemic cell lines and correlated these changes with the differentiative and proliferative status of these cells. We observed that, as in normal cells [9], both oncoproteins decline in differentiating leukaemia cells, well before proliferation ceases in some lineages. Although oncoprotein levels and distribution over the cell cycle are similar among the uninduced cells of different leukaemic lines, oncogene-, cell-

Table 2. Relative $p62^{c\text{-}myc}$ and $p75^{c\text{-}myb}$ and cell cycle status during TPA induction of leukaemic cells to macrophages

Cells	Time					
	0	30 min	2 h	6 h	24 h	48 h
HL60						
C-*myc*	1.0	1.5	2.0	1.4	0.7	0.5
C-*myb*	1.0	1.3	0.95	1.3	1.0	0.85
% S	34.7	39.5	34.1	39.8	15.0	15.3
KG1						
C-*myc*	1.0	1.0	1.3	0.55	0.35	0.4
C-*myb*	1.0	1.1	1.3	0.7	1.4	0.4

Table 3. Relative $p62^{c\text{-}myc}$ and $p75^{c\text{-}myb}$ and cell cycle status during induction of HL60 to granulocytes

Agent	Time						
	0	30 min	2 h	6 h	24 h	48 h	120 h
DMSO							
C-*myc*	1.0	0.7	0.5	0.7	0.5	0.5	0.5[a]
C-*myb*	1.0	1.3	1.1	1.0	0.8	0.6	0.2
% S	32.0	34.1	31.2	31.6	30.1	19.1	7.7
Retinoic acid							
C-*myc*	1.0	1.2	1.2	1.4	1.4	1.2	0.7[b]
C-*myb*	1.0	0.7	0.6	0.8	0.7	0.6	0.3
% S	36.3	35.4	40.0	35.8	29.1	30.9	10.6

[a] 80% NBT-positive cells
[b] 92% NBT-positive cells

Table 4. Relative $p62^{c\text{-}myc}$ and $p75^{c\text{-}myb}$ and cell cycle status in HEL after induction

Agent	Time							
	0	30 min	2 h	6 h	24 h	48 h	72 h	120 h
Hemin								
C-*myc*	1.0	0.8	0.7	2.8	1.1	0.6	0.5	0.6
C-*myb*	1.0	1.1	0.7	1.4	1.0	0.7	0.5	–
% S	39.6	52.0	39.0	40.0	38.0	40.1	39.2	37.3
DMSO								
C-*myc*	1.0	0.7	0.4	0.6	0.3	0.3	0.3	0.2
C-*myb*	1.0	0.7	0.4	0.8	0.4	0.4	0.4	0.3
% S	45.1	37.3	37.3	32.9	30.1	26.5	28.5	32.4

line-, lineage-, and inducer-specific kinetics occur in this decline. The cell cycle distribution of the oncoproteins does not change during maturation. Our data (a) suggest that there is no simple relationship of either oncoprotein to proliferation, (b) confirm other reports [10] that multiple metabolic cascades exist in leukaemic cells to down-regulate and up-regulate genes important in the differentiation process, and (c) support disparate roles for c-*myc* versus c-*myb* during haemopoietic differentiation.

Acknowledgment: This work was supported by the Leukaemia Research Fund, U.K. The Figures and Table 1 are reproduced from Pedrazzoli et al. [11].

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